

# Liparis macrosepala (Orchidaceae), a new species from southwest China with its phylogenetic position

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#### **Abstract**

A new orchid species, *Liparis macrosepala*, is illustrated and described from Yunnan Province, China, based on morphological and molecular analyses. This plant is characterised by the ovoid-fusiform, slightly compressed pseudobulbs with 4 or 5 leaves with slightly crisped margins on their apical half, dorsal sepal heart-shaped, lip with a bituberculate basal callus and a thickened folded lateral lobe on each side, centrally with one cavity with slightly raised margins, the column with a single pair of broadly triangular, obtuse wings. Maximum Likelihood and Bayesian Inference analyses of combined nrITS and plastid *matK* DNA sequences place this species in section *Cestichis*.

#### **Keywords**

Liparis section Cestichis, molecular phylogeny, morphology, matK, nrITS

#### Introduction

The genus *Liparis* Rich. (Epidendroideae, Malaxideae, Malaxidinae) comprises about 320 species distributed worldwide with more than 70 species in China (Pridgeon et al. 1999; Chen et al. 2009; Tian et al. 2015; Huang et al. 2018; Ya et al. 2021). Species

from this genus are terrestrial, lithophytic, epiphytic and rarely mycoheterotrophic, with inflorescences laxly or densely many-flowered, lip often reflexed and usually with a basal callus, lacking a spur, column winged at apex and sometimes at base and four pollinia in two pairs (Chen et al. 2009).

During our field surveys in Xishuangbanna, Yunnan, China, an unknown species was found. In this paper, we analysed the morphological differences of the newly-found species and its allied species and the phylogenetic position of the new entity is also discussed, based on molecular evidence from nrITS and plastid *matK*. After careful morphological comparison and phylogenetic analyses, we concluded that this species is new to science.

## Material and method

## Morphological observations

Materials of the new species were collected from Xishuangbanna, Yunnan, China during a field expedition. Morphological characters were observed, measured and photographed based on five living individuals under a stereomicroscope (SZX16-6151, Olympus, Japan) and photographed with a digital camera (D750, Nikon, Japan). A voucher specimen, designated as the holotype, was deposited at Shanghai Chenshan Herbarium (CSH). Conservation assessment has been conducted following IUCN guidelines (IUCN 2019).

# Taxonomic sampling

DNA sequences of nrDNA ITS and plastid *matK* of the new species were sequenced and sequences of the same markers for 82 related species were downloaded from Gen-Bank, including five outgroup species from other subtribes (Table 1).

# Phylogenetic analyses

DNA sequences were aligned using the MAFFT programme in Geneious v. 2020.2.4 (https://www.geneious.com, accessed on 10 March 2021). Phylogenetic analyses were conducted using Maximum Likelihood (ML) and Bayesian Inference (BI) in RAxML v.7.0.4 (Stamatakis 2006) and MrBayes v.3.2.6 (Huelsenbeck and Ronquist 2001; Ronquist et al. 2012), respectively. The appropriate DNA substitution model under AIC criteria was estimated using jModelTest 2.1.10 (Posada 2008). ML analyses were conducted with bootstrap values calculated by running 1,000 replicates. For BI analysis, four chains were run with random initial trees, each for 1,000,000 generations, until the average standard deviation of the split frequency values was less than 0.01 to ensure convergence, sampling trees every 1,000 generations. After the first 20% of samples were discarded as burn-in, the remaining replicates were used to estimate the posterior probabilities.

**Table 1.** Taxon sampling in this study.

	Species Name	nrITS	matK	
	Acanthophippium mantinianum L.Linden & Cogn.	AF521081	AF263618	
	Collabium simplex Rchb.f.	EF670387	AY557200	
,	Crepidium acuminatum (D.Don) Szlach.	KJ459274	KJ459304	
	Crepidium bahanense (HandMazz.) S.C.Chen & J.J.Wood	MH116611	MH117500	
,	Crepidium bancanoides (Ames) Szlach.	AB290885 AB290		
5	Crepidium brevidentatum (Schweinf.) M.A.Clem. & D.L.Jones	AB290886	AB290894	
7	Crepidium resupinatum (G.Forst.) Szlach.	JN114483	JN004403	
3	Dendrobium dixanthum Rchb.f.	KY966535	KY966825 JN004422	
)	Dienia cylindrostachya Lindl.	JN114491		
0	Eria ferruginea Lindl.	AF521071	AF263660	
1	Eulophia graminea Lindl.	MH768269	MH767976	
12	Liparis macrosepala Z.W. Wang, Y. Zhang & W.C. Huang	ON642332	ON642331 AY907139 KJ459306	
3	Liparis anopheles J.J.Wood	AY907075		
14	Liparis assamica King & Pantl.	KJ459276		
5	Liparis aureolabella J.D. Ya & Z.D. Han	MN065679	MN065733	
6	Liparis auriculata Blume ex Miq.	AB289458	KF262076	
17	Liparis balansae Gagnep1	KF589874	KF589880	
18	Liparis balansae Gagnep2	KJ459277	KJ459307	
19	Liparis bingzhongluoensis X.H. Jin	MW169041	MW169042	
20	Liparis bistriata E.C.Parish & Rchb.f.	KJ459279	KJ459309	
21	Liparis bootanensis Griff	KJ459280	KJ459310 AY907140 AY907165 AY907144	
22	Liparis bracteata T.E.Hunt	AY907076		
23	Liparis brunnescens Schltr.	AY907098		
24	Liparis condylobulbon Rchb.f.	AY907080		
25	Liparis cordifolia Hook.f.	KJ459282	KJ459312	
26	Liparis delicatula Hook.f.	KJ459283	KJ459313	
27	Liparis distans C.B.Clarke	KJ459284	KJ459314	
28	Liparis disticha (Thouars) Lindl.	AY907081	AY907145	
29	Liparis elliptica Wight	KJ459285	KJ459315	
30	Liparis fissilabris Tang & F.T.Wang	KJ459286	KJ459316	
31	Liparis fissipetala Finet	KJ459287	KJ459317	
32	Liparis formosana Rchb.f.	AY907082	AY907147	
	Liparis fujisanensis F.Maek. ex Konta & S.Matsumoto	EU024936	EU024937	
33 34				
	Liparis gibbosa Finet-1	AY907083	AY907148	
35	Liparis gibbosa Finet-2	AY907084	AY907149	
36	Liparis glossula Rchb.f.	KJ459289	KJ459319	
37	Liparis guangxiensis C.L.Feng & X.H.Jin	KF589875	KF589881	
38	Liparis japonica (Miq.) Maxim.	AY907086	AY907151	
39	Liparis koreana (Nakai) Nakai	EU017422	EU017444	
40	Liparis kumokiri F.Maek.	AY907087	AY907152	
<b>41</b>	Liparis latifolia Lindl.	AY907088	AY907153	
42	Liparis latilabris Rolfe	KJ459291	KJ459321	
43	Liparis liliifolia (L.) Rich. ex Lindl.	AY907090	AY907156	
44	Liparis loeselii (L.) Rich.	AY907091	AY907157	
45	Liparis makinoana Schltr.	EU017405	EU017428	
16	Liparis mannii Rchb.f.	KJ459293	KJ459323	
<b>i</b> 7	Liparis meihuashanensis S.M.Fan	MF959772	MF959773	
<b>18</b>	Liparis mengziensis J.D. Ya & Lei Cai	MN065734	MN065678	
19	Liparis nanlingensis H.Z.Tian & F.W.Xing	AB701346	/	
60	Liparis napoensis L.Li, H.F.Yan & S.J. Li-1	MT012899	MT019986	
51	Liparis napoensisL.Li, H.F.Yan & S.J. Li -2	MT012900	MT019987	
52	Liparis nervosa (Thunb.) Lindl.	AY907092	AY907158	
53	Liparis nugentiae F.M.Bailey	AY907093	AY907159	
54	Liparis odorata (Willd.) Lindl.	KJ021033	KJ021029	
55	Liparis pandurata Ames	AY907094	AY907160	
56	Liparis pauliana HandMazz.	AY907096	AY907163	

	Species Name	nrITS	matK	
57	Liparis petiolata (D.Don) P.F.Hunt & Summerh.	MW186826	MW187482	
58	Liparis resupinata Ridl.	KJ459297	KJ459327	
59	Liparis somae Hayata-1	MT012898	MT019985	
60	Liparis somae Hayata-2	MT012897	MT019984	
61	Liparis sootenzanensis Fukuy.	KJ021034	KJ021030	
62	Liparis stricklandiana Rchb.f1	MT012903	MT019990	
63	Liparis stricklandiana Rchb.f2	KJ459298	KJ459328	
64	Liparis sula N.Hallé	AY907104	AY907171	
65	Liparis terrestris J.B.Comber	AY907105	AY907172	
66	Liparis truncicola Schltr.	AY907106	AY907173	
67	Liparis viridiflora (Blume) Lindl1	MT012902	MT019989	
68	Liparis viridiflora (Blume) Lindl2	MT012901	MT019988	
69	Malaxis brachypoda (A.Gray) Fernald	AY907108	AY907175	
70	Malaxis monophyllos (L.) Sw.	MW181626	MW187483	
71	Malaxis soulei L.O.Williams	AY907119	AY907186	
72	Malaxis abieticola Salazar & Soto Arenas	AY907129	AY907196	
73	Oberonia acaulis Griff.	KY242066	KY241943	
74	Oberonia brunoniana Wight	JN114623	JN004516	
75	Oberonia equitans (G.Forst.) Mutel	AY907130	AY907198	
76	Oberonia heliophile Rchb.f.	AY907131	AY907199	
77	Oberonia iridifolia Roxb. ex Lindl.	AY907132	AY907200	
78	Oberonia mucronata (D.Don) Ormerod & Seidenf	JN114640	JN004534	
79	Oberonia neocaledonica Schltr1	AY907133	AY907201	
80	Oberonia neocaledonica Schltr2	AY907134	AY907202	
81	Oberonia padangensis Schltr.	AY907135	AY907203	
82	Oberonia wappeana J.J.Sm.	AY907138	AY907206	
83	Oberonioides pusillus (Rolfe) Marg. & Szlach.	KJ527610	KJ459302	

## Results

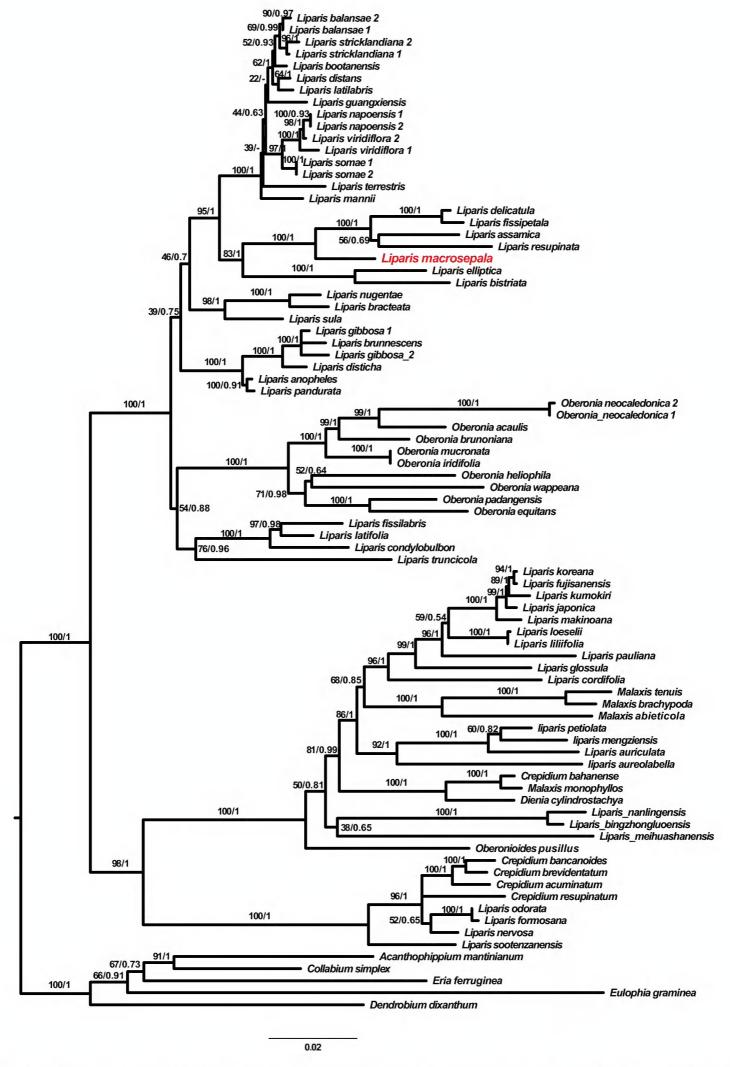
# Phylogenetic analyses

The length of nrITS matrix was 792 bp including 262 parsimony-informative sites and for *matK*, the length and parsimony-informative sites were 1443 bp and 120, respectively. Both analyses (MP and BI) recovered similar relationships. The ML tree with bootstrap percentages, on which the posterior probabilities from the BI analysis were also indicated, is shown in Fig. 1.

The phylogenetic analyses indicate that *Liparis* is not monophyletic, being mingled with species of other genera of Malaxideae. This result agrees with what was found in previous studies (Cameron 2005; Margońska et al. 2012; Tang et al. 2015; Li et al. 2020; Kumar et al. 2022). The new species, henceforth referred to as *Liparis macrosepala* Z.W. Wang, Y. Zhang & W.C. Huang, is grouped with species in *Liparis sect. Cestichis* Thouars ex Pfitzer as the sister of a clade consisting of *L. delicatula* Hook.f., *L. fissipetala* Finet, *L. assamica* King & Pantl. and *L. resupinata* Ridl.

# Morphological comparisons

Liparis is defined as species with racemose inflorescences, resupinate lip lacking a spur, column without a conspicuous foot and four pollinia in two pairs with small viscidium, but no caudicle. The morphology of Liparis macrosepala is in



**Figure 1.** Maximum Likelihood tree of *Liparis* and its allied genera in subtribe Malaxidinae inferred from the combined analysis of nrITS and *matK*. ML bootstrap values (ML<sub>BP</sub>)/Bayesian posterior probabilities (PP) are indicated above the branches, respectively. The sectional taxonomy of *Liparis* follows Garay and Romero-Gonzalez (1999) and Li et al. (2020).

accordance with the characteristics of sect. *Cestichis* like the slightly flattened, narrowly winged rachis with alternating bracts. The morphological characters can distinguish *Liparis macrosepala* from its close relatives *L. delicatula*, *L. fissipetala*, *L. assamica* and *L. resupinata*.

### Taxonomic treatment

Liparis macrosepala Z.W. Wang, Y. Zhang & W.C. Huang, sp. nov.

urn:lsid:ipni.org:names:77306143-1

Figs 2, 3

Chinese name: 大萼羊耳蒜

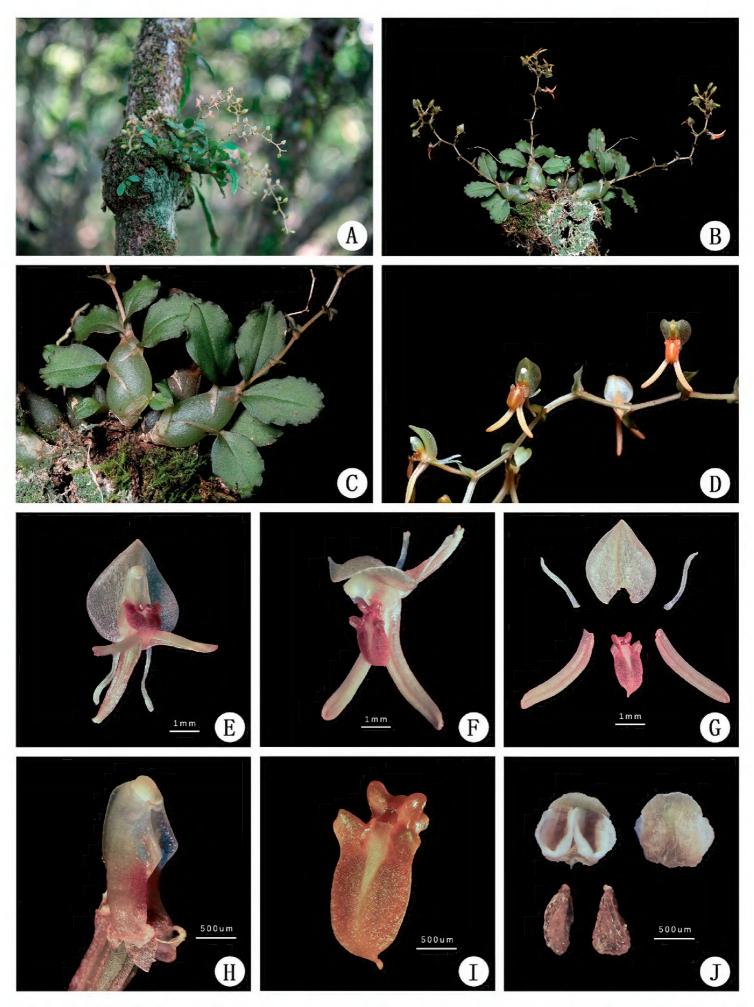
**Type.** China. Yunnan Province (云南), Xishuangbanna (西双版纳), Mengla County (勐腊县) epiphyte on the tree trunk, 1620 m elev., 23Nov 2021, Zhengwei Wang, Xiaochen Li, Yu Zhang& Zhijin Wu, WZW04247 (holotype: CSH!)

**Diagnosis.** *Liparis macrosepala* is characterised by the ovoid-fusiform, slightly compressed pseudobulbs with 4 or 5 alternate leaves on their apical half, these with slightly crispate margins, dorsal sepal ovate with cordate base, broadly elliptic, ca. 4 mm long, 2 callus-shaped and thickened folds, base with 2 oblong lobes on both sides, centrally with 1 thickened, concave callus, column with a single pair of arcuate wings.

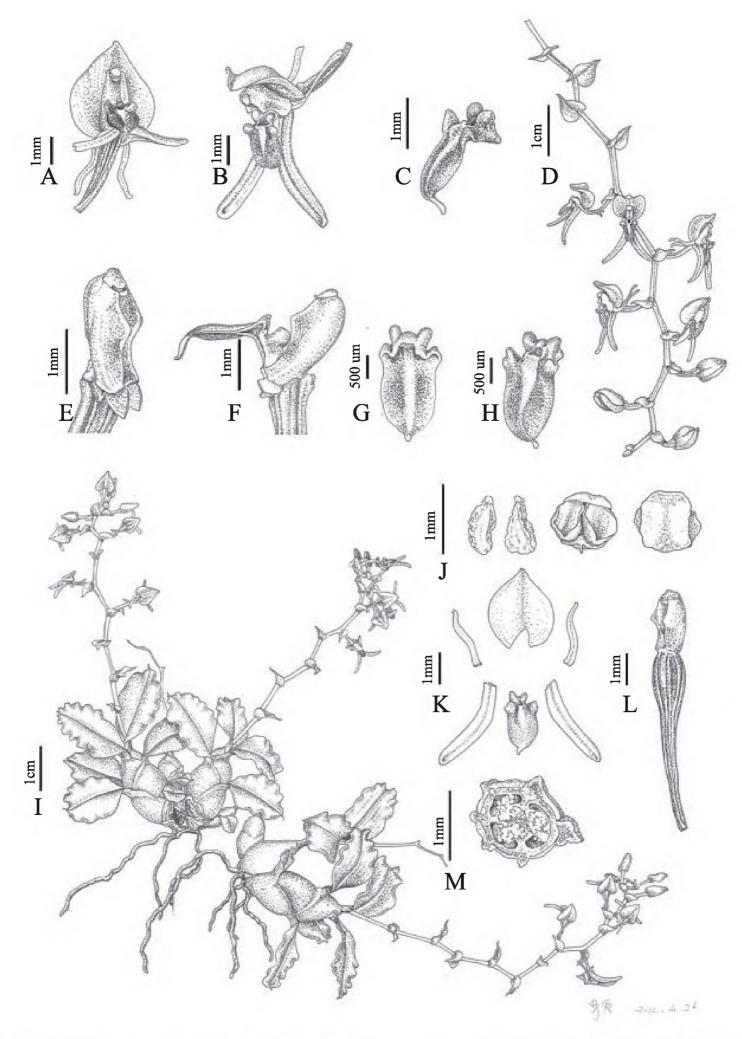
Epiphytic herbs. Roots slender, flexuose. Pseudobulbs clustered, ovoid-fusiform, slightly compressed laterally,  $1-2 \times 0.5-1$  cm, upper half with 4–5 widely spaced leaves. Leaf blade ovate-oblong,  $1.8-2.3 \times 0.8-1.2$  cm, apex acuminate, base contracted into a short petiole, articulate, margins of their apical half slightly crispate. Peduncle 7–10 cm long, with several sterile bracts 2-5 mm long; raceme with 7-10 flowers arranged in zigzag manner. Floral bracts broadly ovate with cordate base,  $2-3 \times 1-1.5$  mm, acute. Flowers greenish-orange; pedicel and ovary ca. 7 mm long. Dorsal sepal broadly ovate with cordate base,  $3.2-5 \times 3-3.6$  mm, 1-veined, abaxially carinate, apex acute; lateral sepal oblong-ovate or ovate-lanceolate,  $5-6 \times ca$ . 0.6 mm long, abaxially slightly carinate. Petals narrowly linear,  $3-4 \times \text{ca. } 0.2 \text{ mm}$ ; lip elliptic,  $2-3 \times \text{ca. } 1 \text{ mm}$ , apex apiculate, base bearing a bituberculate callus, then expanded on each side into a thickened, folded, rounded lobe, with 1 excavation with raised margins between the lobes. Column straight, ca. 2 mm long, with a pair of subtriangular, obtuse wings on each side near the middle and a ridge on the back of the column. Anther cap hemispherical, pale yellow; pollinia 4 in 2 pairs with one pollinium of each pair smaller than the other, waxy, brownish, with minute apical viscidium.

Phenology: Flowering in November–December.

**Distribution and habitat.** It is found on tree trunks on a limestone ridge-top evergreen broad-leaved forest at an elevation of 1500–1700 m in Mengna County, Xishuangbanna Autonomous Prefecture, Yunnan Province, People's Republic of China. The habitat presents a tropical monsoon climate.



**Figure 2.** Morphology of *Liparis macrosepala*. **A** plants in situ **B** flowering plant **C** pseudobulbs and leaves **D** inflorescence **E** flowers, front view **F** flowers, side view **G** perianth dissection **H** column from side **I** lip in oblique view **J** anther cap and pollinia. Photographs by Weichang Huang.



**Figure 3.** *Liparis macrosepala* **A** flower, front view **B** flower, side view **C** lip, side view **D** inflorescence **E** column, side view **F** lip and column, side view **G** lip, back view **H** lip, front view **I** flowering plant **J** pollinia and anther cap **K** perianth dissection **L** column and ovary, oblique view **M** ovary, transection. Drawn by Lan Yan.

**Etymology.** The species epithet refers to the large and conspicuous dorsal sepal of the flower.

**Taxonomic notes.** Liparis macrosepala differs from L. delicatula in its 4 to 5 leaves with slightly crispate margins on their apical half and single pair of wings on the column. Its entire, not Y-shaped petals and sessile lip (i.e. without a claw) easily distingush L. macrosepala from L. fissipetala. The dosal sepal of L. assamica is narrowly ovate-oblong, in contrast with the heart-shaped dorsal sepal of Liparis macrosepala. Liparis resupinata is distinguished from L. macrosepala by its 10–50-flowered raceme and the column with a single pair of broad wings, each with a retrorse thread. The main differences between these closely-related species, according to our phylogenetic analyses, are summarised in Table 2.

**Conservation assessment.** The new species was found in a ridge-top evergreen broad-leaved forest on a limestone mountain. Despite numerous surveys in the areas, only six mature individuals were found without fruits or evidence of cross-pollination.

This extremely small effective population occurs in a touristic zone which is a serious threat to the survival of the species. Consequently, the species can be assessed as Critically Endangered (CR, D), based on current information and following IUCN guidelines (IUCN 2019).

<b>Table 2.</b> Comparison of <i>L. macrosepala</i> and re	elated s	pecies.
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Characters	L. delicatula	L. fissipetala	L. assamica	L. resupinata	L. macrosepala
Pseudobulbs	oblong or	ovoid, 8–10 mm	ovoid-fusiform, slightly	subcylindrical or ±	ovoid-fusiform, slightly
	cylindrical-fusiform	long	compressed 1.5-2.5	spindle-shaped, 1.8-5	compressed, 1–2 cm ×
	5–9 3–5 mm		cm × 6–10 mm	$cm \times 3-6 mm$	0.5–1 cm
Leaf	2 or 3, margin flat	3 or 4, strongly	3 or 4, apical half	3 or 4, margin slightly	4 or 5, apical half slight-
		crisped-margined	slightly crisped- margined	serrate	ly crisped-margined
Scape	2–5 cm, several to	5–10 cm long, with	10–13 cm, more than	7–18 cm, 10–50-flow-	7–10 cm, more than
	10-flowered, flowers	10-15 flowers, flow-	10-flowered, flowers	ered, flowers pale green	10-flowered, flowers
	white	ers yellow,	orange	or greenish-yellow	greenish-orange
Bracts	ovate-lanceolate,	ovate-lanceolate,	lanceolate, 2-3 mm	lanceolate, 3–5 mm	broadly ovate, 2-3 mm
	2–3 mm	1.5–3.5 mm			
Dorsal sepal	ovate-oblong, 2.5–3	oblong-lanceolate,	narrowly ovate-oblong,	oblong or elliptic-ob-	broadly ovate, ca. 3.2-5
	× 1.5–1.8 mm	$3-4 \times 0.8-1 \text{ mm}$	$4.8-5.8 \times \text{ca. } 1.6 \text{ mm}$	long, ca. $4 \times 1.8$ mm	$\times 3-3.6$ mm
Petals	narrowly linear-	narrow linear, 4-5	narrowly linear, 5–5.5	narrowly linear, ca. 3.5 ×	narrowly linear, 3–4 ×
	lanceolate, $2.5-3 \times$	mm long, Y-shaped	× ca. 0.7 mm, entire	0.3 mm, entire	ca. 0.2 mm, entire
	ca. 0.5 mm, entire				
Lip	broadly elliptic or	epichile broadly ob-	broadly obovate-ob-	broadly elliptic-oblong	broadly elliptic, ca. 2–3
	orbicular, ca. 2.5	long or subsquare,	long, ca. $4 \times 2.7$ mm,	or broadly ovate-oblong,	mm long, two callus-
	mm, base with	$1.5 - 2 \times 1 - 1.5$	with two callus-shaped	2.5–3 mm, with two	shaped and thickened
	an orbicular, auricu-	mm, base with two	thickened folds, two	lateral splits below mid-	folds, base with two
	late, callus-shaped	auricles on both	suborbicular lobes on	dle; two suborbicular	oblong lobes on both
	fold on either side,	sides; claw short,	both sides, centrally	lobes, centrally with one	sides, centrally with
	with a concave cal-	with a fleshy callus	with one concave cal-	bilobed callus near base	one bituberculate callus
	lus near base	centrally near base	lus near base		near base
Column	ca. 2.2 mm, two	ca. 1.5 mm,	ca. 2 mm, two pairs	ca. 2.8 mm, a pair of	ca. 2 mm, a single pair
	pairs of wings	broadly winged	of wings	wings, each with a	of subtriangular wings
		with two horn-like		retrorse thread	
		appendages			

## **Acknowledgements**

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